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EFFECTS OF SOME SINGLE VIRUSES AND OF COMBINATIONS OF THE SAME VIRUSES ON THREE POTATO VARIETIES.

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(Plates 10 and 11).

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Introduction.

Notwithstanding the numerous papers which have appeared on the effects of mosaic on the yield of potatoes, so far as the authors are aware, there is no detailed account of the effect of virus A or of Crinkle, virus (X+A), on the yield. The present article records experiments on the effects of definite strains of virus X and of virus A on three potato varieties, together with the production of Crinkle by these viruses and its effect on the same potato varieties.

Virus X. This is the commonest virus occurring in potatoes throughout the world, and is the cause of simple mosaic. Despite all the investigations carried out on this virus, no insect vector of it is yet known, and, up to the present, the only method of spread of virus X which has been demonstrated has been by contact of diseased with healthy plants (4). In the field spread of infection is limited to tolerant varieties of potatoes, and where intolerant varieties are grown alongside virus-infected plants no spread of disease occurs.

Numerous strains of virus X are known to exist, ranging in virulence from those carried symptomlessly in potatoes (latent mosaic) through all stages of visible mottle

to severe forms which seriously reduce the size and vigour of affected plants, and result in diminished yield. Previous to 1946 it was also generally claimed, that even masked strains of virus X caused a reduction in yield of at least 12 per cent. In that year, critical experiments carried out at the Albert Agricultural College, Glasnevin, with two latent strains of virus X occurring naturally in the variety Up-to-Date showed that under Irish conditions no such reduction in yield took place, (2). Full confirmation of this was forthcoming two years later when the experiments were repeated, (3). Not only was there no reduction in yield caused by the latent strains of virus X, but occasionally the diseased plants gave a slightly higher yield than the virus-free plants, (2). Potato varieties which carry a latent strain of virus X are immune to further infection with severe strains of this virus, and, in so far as a latent strain exercises this protective role, the immunity conferred by it is beneficial.

Now, latent strains of virus X do not spread to nor wipe out intolerant varieties under field conditions ; there is no reduction in yield caused by these latent strains of the virus, and their presence in tolerant potato varieties protects the plants from infection with severe strains of virus X. Why then their importance ? For the simple reason that no immunity is acquired against infection with a different virus, and the presence of latent X in a plant is potentially dangerous, for, should infection with a different virus take place, the effect on both foliage and yield is serious.

Virus A. This is an insect-transmitted virus, which is common in many old potato varieties. When present alone, the effect of virus A on the foliage is somewhat similar to that of mild strains of virus X. Plants showing symptoms of virus A cannot be identified with certainty in the field from those infected with mild mosaic, although the mottle is, as a rule, rather more diffuse.

Crinkle, Virus ($X+A$). The composite nature of this disease was first shown by Murphy and McKay in 1931–32, (5 & 6). Whilst latent strains of virus X or virus A alone cause negligible effects on the foliage, or at the most a slight transient mottle, the disastrous effects of the two together produce Crinkle. The characteristic features of this malady are the severe puckering and reduction in size of the leaflets, and the occurrence of pale or yellowish blotches which give a conspicuous mosaic effect. The chlorotic tissues fail to develop to the same extent as the green areas, so that bulging of the lamina results in a striking irregularity of the leaf surface. Frequently the foliage is so distorted as to render the variety unrecognizable. Although not visibly affected, the tubers are reduced in size and number, and, just as in the case of the single viruses, both viruses are invariably perpetuated by the tubers, and these give rise to Crinkle plants. The constituent or associated viruses of this disease were investigated by Clinch and Loughnane (1). They showed that, in addition to producing Crinkle by putting simple mosaic into plants already having virus A, or *vice versa*, the mixture of viruses could also be separated, virus X being obtained by means of needle inoculation, and virus A by means of the aphid *Myzus persicae* (1). Owing to the difference in the mode of transmission of the two viruses concerned, two separate infections are necessary for the production of Crinkle in perfectly healthy plants.

Material and Preparatory Work.

In previous work by Clinch and McKay (2 & 3), the latent strains of virus X carried by the variety Up-to-Date were referred to as strain (*a*) and (*b*) respectively. Two quite different strains of virus X were worked with in the present experiments, and to prevent any confusion hereafter, these strains are designated as virus X*c* and virus X*d*.

Virus Xc. This is the mildest strain of so-called potato mosaic ever encountered in the work at Glasnevin. Five years ago, during the ordinary course of testing plants for health, virus X*c* was detected in the variety Stormont Dawn. It was repeatedly met with in this variety, where it seems to be of general occurrence. No effects whatever are produced by this virus on Stormont Dawn, and on inoculation into other potato varieties tolerant of virus X, it is also carried symptomlessly. Even when virus X*c* is inoculated into indicator plants of *Datura stramonium*, the reaction of this host is so slight that the presence of a virus could easily be overlooked, only a few small necrotic spots appearing on the leaves. However, as will be shown later, when virus X*c* is put into plants already carrying virus A, typical Crinkle develops.

Virus Xd. This strain of potato mosaic was isolated by means of needle inoculation from Crinkle plants by Clinch and Loughnane in 1932 (1). It was added to the existing stock of viruses at that time, and it has been so maintained ever since at Glasnevin, mainly in the variety President.

Virus A. This particular virus has been in stock at Glasnevin since 1924, chiefly in the variety Irish Chieftain.

Virus X*c*, virus X*d*, and virus A all appear to be remarkably stable, as during the time each of them has been kept going in potato varieties in the glasshouse at Glasnevin, there has never been any indication of a permanent change occurring in any one of them.

The three potato varieties selected for the experiment were Arran Banner, Majestic, and President. The two first mentioned are early maincrops, whilst the third is a late maincrop. Each of these varieties was already known to be tolerant to many strains of virus X and also to virus A. Furthermore, Crinkle plants are of frequent occurrence in each of these varieties under ordinary farming conditions.

Early in the year 1950 a large tuber of virus-free Arran Banner was cut into six parts. This eliminated all question of any possible clonal difference likely to occur afterwards. The six pieces were then planted in pots in the glasshouse. Whilst the plants were still young two of them were grafted with scions carrying virus X*c*, two with scions carrying virus X*d* and one with virus A. The sixth plant was grown as a control in a separate house. Later in the growing season, one of those plants previously grafted with virus X*c* was again top grafted with a scion carrying virus A; and one of the pair previously grafted with virus X*d* was treated in a similar manner. Exactly the same procedure was carried out with Majestic and President.

In order to obtain sufficient tubers for reasonably sized yield tests, propagation of the various groups was carried out in four plots situated in a large field of grain in 1951. These plots were isolated from each other by from 300 to 500 yards. One plot contained the produce of all plants previously grafted with virus X*c* and with

virus X*d*. As these strains are mutually protective, it was not considered necessary to separate them. Similarly, another plot contained the Crinkle plants, virus (X*c*+A) and virus (X*d* + A). A third plot contained the produce of plants grafted with virus A ; whilst the propagation of tubers from the healthy controls was carried out in the remaining plot, together with plants of Immune Seedling, which were grown to provide buffer drills the following year, where these were considered necessary.

Yield Trials 1952.

The experimental plot was situated in an ordinary field of potatoes. The plot was bordered along each side by four drills of virus-free potatoes, and each drill had two extra plants at each end which were discarded when the plot was dug. In addition, there was a vacant space six yards wide between the ends of the drills and the other potatoes in the field, the other ends of the drills being on the edge of a road.

Arrangement of Drills in Field Plot 1952.

Drill 1	Arran Banner	Healthy	50	Plants	
2	Majestic	Healthy	50	,,	
3	President	Healthy	50	,,	
4	Immune Seedling				
5	Arran Banner	Virus X*c*	45	,,	
6	Majestic	Virus X*c*	50	,,	
7	President	Virus X*c*	50	,,	
8	Arran Banner	Virus X*d*	30	,,	
9	Majestic	Virus X*d*	50	,,	
10	President	Virus X*d*	50	,,	
11	Immune Seedling				
12	Arran Banner	Virus (X*c* + A)	20	,,	and
	President	Virus (X*c* + A)	30	,,	
13	Majestic	Virus (X*c* + A)	50	,,	
14	Arran Banner	Virus (X*d* + A)	18	,,	and
	President	Virus (X*d* + A)	32	,,	
15	Majestic	Virus (X*d* + A)	50	,,	
16	Immune Seedling				
17	Arran Banner	Virus A	44	,,	
18	Majestic	Virus A	50	,,	
19	President	Virus A	50	,,	

Manuring was at the rate of 20 tons farmyard manure plus 7 cwt. artificials (potato manure) per statute acre, the dung being placed in the bottom of the drills.

Planting was done on 7th April in drills 30 inches apart with the sets 18 inches apart in the drill. During the remainder of the season the plot received the ordinary cultivation given to the remainder of the field.

Climatic conditions. The rainfall during the early months of 1952 was somewhat below normal, and the summer was also rather dry as will be seen from the following figures, which represent the rainfall in inches for each month in the growing season ; April 1·69, May 2·26, June 1·32, July 0·87, August 3·29, September 2·64. Weather conditions throughout were very favourable for the potato crop. As the weather threatened to break towards the end of June the plot was sprayed with a 2 per cent. Burgundy mixture on 27th June. Potato blight, *Phytophthora infestans*, had already made its appearance elsewhere in the country, and as a matter of interest it had been identified in Co. Dublin on 27th May, the disease being found by Mr. Loughnane on potato plants growing on a heap of discarded tubers. The threatened break in the weather did not occur immediately, but, instead, it became very sultry and warm up to 6th July, when the first rain of any consequence for some time fell. As the remainder of July was dry the plot was not again sprayed until the 30th July, when a second application of a 2 per cent. Burgundy mixture was given. Potato blight was present on adjoining potatoes on 1st September, and the first affected foliage in the plot was found on 5th September. Blight, however, on the experimental plot was a negligible factor throughout, and at digging time when the potatoes were dug and graded, only seven blighted tubers occurred.

Effects of the Different Viruses on the Tops and Foliage.

These observations were made on 23rd June, when the plants were just beginning to flower and before spraying was done. The general appearance of the individual groups thereafter did not undergo any substantial change until the plants started to mature. The effects of the various viruses and combinations are best described under variety headings.

Arran Banner.

No symptoms of any disease whatever could be detected in plants of this variety carrying any of the single viruses X*c*, X*d* or A, and all of these would have passed for healthy in the field.

Crinkle, virus (*Xc* + *A*). Plants carrying this mixture were slightly reduced in height, with the stems somewhat weak and a tendency to sprawl. The foliage had a curious grey appearance and an obvious mottle.

Crinkle, virus (*Xd* + *A*). The weakness of the stems and sprawling habit of the plants very definite. The leaflets badly mottled, wavy at the margins and much reduced in size, especially on the top of the plant, see Fig. 4, Plate 11.

Majestic.

As in the previous variety, no symptoms of disease in plants carrying single viruses X*c*, X*d*, and A, and all would have passed for healthy plants.

Crinkle, virus (*Xc* + *A*). An obvious mottle present in all plants, but not so well defined as in corresponding plants of Arran Banner. The general appearance

of these Majestic plants suggested a strong mosaic rather than a typical Crinkle, as there was no puckering of the laminae.

Crinkle, virus (*Xd* + *A*). Stems weak and plants sprawling over the drills. Mottling and puckering of the leaves well developed as was also the waviness of the margins. Leaflets on top of the plant showed the worst effects. The general appearance of the effect of the mixture on foliage of Majestic is shown in Fig. 5, Plate 11.

President.

Virus Xc. Plants with this virus all have a harsh appearance, and the green colour is not so good as in that of healthy President. There is nothing very definite in the appearance of the plants, but they would be queried as to their state of health.

Virus Xd. A mosaic showing in every plant. Although the mottle is not obvious in every leaf, it is clearly seen in the centre foliage, and is very definite. None of these plants would pass for healthy.

Virus A. President is the only variety carrying this virus which might be questioned for its state of health. There is a harsh colour in the plants which is not a normal appearance of President foliage. A few of the plants show a diffuse mottle here and there but no such mosaic is apparent in others.

Crinkle, virus (*Xc* + *A*). Plants reduced in height and stems showing freely owing to reduced foliage. Leaflets badly mottled with yellowish blotches, somewhat reduced in size, puckered, and with wavy margins. The effects of the mixture of viruses was much worse than on the two other varieties with the same mixture. Plants typical Crinkle, Fig. 2, Plate 10.

Crinkle, virus (*Xd* + *A*). Plants somewhat worse than those with (Xc + A), but the difference is one of degree rather than of kind. Stems spindly and bare. As this variety is one of very erect growth there was no sprawling however. Big reduction in size of leaves and of leaflets. The terminal leaflet was often distorted or kinked and its petiole was very short. The leaflets were badly puckered, very wavy at the margins, and had a well developed yellowish mottle. Some brown necrotic spots on the upper surface of the foliage. The effects of this mixture on the foliage is well illustrated in Fig. 3, Plate 11.

As the reduction in size of the foliage, especially in President, from both types of Crinkle was so severe that it left the stems bare and exposed to the observer's view, whereas in the healthy plants the stalks were practically hidden by the mass of foliage, it was decided to take some measurements of diseased and healthy leaves. For this purpose the third leaf up from the base of the stalk was selected, as it was well developed and least likely to be damaged by cultural operations. Measurements were taken from the base of the petiole to the tip of the terminal leaflet. The result in the case of President was so striking that measurements were then taken for all groups in the different varieties. Fifty leaves were measured, one from each plant, except in those cases where less than 50 plants were available, when the third basal leaf on several stems of each plant was measured to make the total 50. These measurements are presented in the following Table.

Table 1.

Mean Length in inches of 50 Leaves in the Different Groups in Each Variety.

State of Health	Arran Banner	Majestic	President
Healthy	13·1	13·8	11·8
Virus X*c*	13·8	13·6	11·3
Virus X*d*	13·0	13·0	10·5
Virus A	12·4	12·7	10·7
Crinkle			
Virus (X*c* + A)	12·1	12·0	9·3
Virus (X*d* + A)	11·2	11·6	8·7

Mention has already been made of some days of very warm sultry weather which followed the spraying on 27th June. No ill effects of the spray were noticed until after rain fell on 6th July, when almost immediately, some scorching of the tips of the foliage developed on the Crinkle plants but not on any of the other groups. Furthermore, during very warm days when the plants in the field showed some flagging, wilting of Crinkle plants was more obvious than in any of the other lots. By the 20th September the Crinkle plants of all varieties were prematurely withered, though the other groups were still green. The plants containing virus A ripened off slightly before the remainder. The tubers were dug on 17th October, bagged, taken into a glasshouse, where they were freed from dirt, and graded into ware, seed and chats, as shown in Table 2.

Table 2.

Comparative Yields of Healthy and Virus-Infected Plants, 1952.

Arran Banner.

State of Health	No. of plants	Ware lb.	Seed lb.	Chats lb.	Total Yield lb.	Av. Yield per plant lb.	Yield as Percentage of Healthy
Healthy	50	194	43	23	260	5·2	100
Virus X*c*	45	194	28	24	246	5·4	103·8
Virus X*d*	30	105	23	17	145	4·8	92·3
Virus A	44	148	30	18	196	4·4	84·6
Crinkle							
Virus (X*c* + A)	20	68	10	8	86	4·3	82·6
Virus (X*d* + A)	18	38	11	3	52	2·8	53·8

MAJESTIC.

State of Health	No. of plants	Ware lb.	Seed lb.	Chats lb.	Total Yield lb.	Av. Yield per plant lb.	Yield as Percentage of Healthy
Healthy	50	147	39	23	209	4·1	100
Virus X*c*	50	108	73	36	217	4·3	104·8
Virus X*d*	50	117	61	35	213	4·2	102·4
Virus A	50	151	36	27	214	4·2	102·4
Crinkle							
Virus (X*c* + A)	50	140	30	25	195	3·9	95·1
Virus (X*d* + A)	50	67	34	28	129	2·5	60·9

PRESIDENT.

Healthy	50	65	90	62	217	4·3	100
Virus X*c*	50	76	84	58	218	4·3	100
Virus X*d*	50	56	64	70	190	3·8	88·3
Virus A	50	99	44	41	184	3·6	83·7
Crinkle							
Virus (X*c* + A)	30	46	23	21	90	3·0	69·7
Virus (X*d* + A)	32	18	31	12	61	1·9	44·1

DISCUSSION.

Where the latent strain of mosaic virus X*c* was carried symptomlessly in Arran Banner, Majestic, and President, there was no reduction whatever in yield. Not only so, but in two of the varieties the yield of the infected plants was slightly higher than that of the virus-free controls. These results are in perfect agreement with those previously recorded from this Department for other latent strains of virus X in the variety Up-to-Date (2 and 3).

In the case of virus X*d* some reduction in yield occurred in Arran Banner, but as it is less than 10 per cent. it is probably not significant. Under field conditions little importance can be attached to differences of less than 10 per cent., unless they occur consistently under repeated trials. In the variety Majestic the yield of diseased plants was slightly above that of the healthy plants. In President, however, where virus X*d* produced a definite mottle in the foliage and the mean length of the leaf was reduced by approximately an inch, the yield was down by over 11 per cent.

Plants infected with virus A, as already stated, ripened off somewhat prematurely, and the reduction in yield in Arran Banner and in President was approximately 15 and 16 per cent., respectively. During the growing season, neither of these varieties had shown any recognizable symptoms of mosaic, although the foliage of President was not above suspicion. It was known from past experience in the glasshouse that with President infected with virus A a diffuse transient mottle frequently developed. This mottle was evidently suppressed in the field and was replaced by a hard, harsh appearance in the foliage, which was not normal to the variety. Virus A was carried symptomlessly by Majestic, and here again there was no reduction in yield.

Considering the extreme mildness of virus X*c*, the most surprising thing in these experiments was the serious Crinkle which developed where virus A was put into plants carrying this latent X. The effect was to reduce the yield in Arran Banner and in President by 17 and 30 per cent., respectively. The effect of this particular mixture on Majestic was not nearly so severe on the foliage as it was on the other two varieties, and, if differences of less than 10 per cent. are not significant, then the effect of virus (X*c* + A) on the yield of Majestic must be considered negligible.

It is seen that where virus A and the very mildest strain of virus X occur in a plant together the mixture gives rise to Crinkle in Arran Banner and in President. This method provides a very easy means of testing plants for latent strains of virus X, that is grafting them unto plants which already have virus A. Plants of President and of Irish Chieftain having virus A are most suitable for this purpose, much more suitable than indicator plants like *Datura stramonium*. If desired, grafting can be dispensed with, and sap inoculation used instead, but the writers prefer grafting, as only a single plant with virus A is required to give positive results.

The real danger of latent strains of virus X lies, not in the reduction in yield of carrier varieties as Smith and Markham claimed (7), but in the potential danger which ensues where an insect transmitted virus like virus A is liable to get into such plants. It is well known, that the ubiquitous virus X is one of the commonest components of mixed virus diseases occurring in potatoes.

Crinkle produced by virus (X*d* + A) was the most severe form of disease on all varieties. In addition to severe mottling, puckering and reduction in size of the leaflets, the mean length of the leaf was reduced by from 2 to 3 inches, and corresponding reductions in yield were of the nature of 46, 29 and 55 per cent. respectively in Arran Banner, Majestic and President.

Throughout the experiments, it will be seen that Majestic suffered less both in foliage and yields from the different viruses and combinations than the other two varieties, and Arran Banner somewhat less than President. These are evidently varietal differences. The effects of both forms of Crinkle on Arran Banner and President were very severe. This had already been obvious from the yields of the propagation plots in 1951, as sufficient tubers were not produced that year to make the requisite number of 50 available for the yield trials in 1952.

One feature which was outstanding in the different groups in 1952 was the uniformity of each lot of plants. In fields of varieties subject to Crinkle, viz. Arran

Chief, Champion, Majestic, and Golden Wonder, the infected plants vary greatly in the amount of mottling, distortion and reduction in size of leaflets. The appearance of the diseased crop does not, as a rule, change substantially from one year to another. The present experiment would indicate that such variation in symptoms in a crop is probably due to different strains of virus X which are present in combination with virus A. Different strains of virus A may, of course, also occur, but no evidence of such has been yet forthcoming.

Acknowledgments are due to Professor M. Caffrey who granted permission for the propagation of the various lots in isolated plots in grain crops in 1951, and we are indebted to Mr. P. J. Barry (late of the Division of Plant Pathology) for his help in various cultural work with the plots both in 1951 and 1952.

Summary.

The effects on three potato varieties of two strains of virus X and of the insect-transmitted virus, virus A, alone and in combinations, are recorded.

One of the strains of virus X was an extremely mild form carried symptomlessly in potatoes, and the other a somewhat more severe type usually producing a definite mottle in potato foliage.

Any possible clonal differences were eliminated by using a large single tuber of each of the varieties, Arran Banner, Majestic, and President. In 1950 each tuber was cut into six pieces, which were then planted in the glasshouse. One plant of each variety was set aside as a control, and the other five grafted with scions carrying the different viruses, some plants being double grafted. The progeny of these plants was propagated in isolated plots situated in a field of grain in 1951, and a yield test was carried out in 1952.

No reduction whatever in yield was caused by the mild strain of virus X which was carried symptomlessly by all varieties.

The more severe strain of virus X caused a slight reduction in yield of Arran Banner, which, however, was not significant; it was carried as latent mosaic in Majestic without causing any reduction in yield, but in President this strain of virus X produced a definite mottle in the foliage and reduced the yield by over 11 per cent.

Virus A caused a reduction in yield in Arran Banner and President of approximately 15 and 16 per cent., respectively; this virus was carried symptomlessly by Majestic without causing any reduction in yield.

Crinkle produced by a combination of the mild strain of virus X and virus A reduced the yields in Arran Banner and President by 17 and 30 per cent. respectively. Although a strong mosaic was produced on the foliage of Majestic by the same Crinkle, the effect on the yield was apparently negligible.

Crinkle produced by combining the more severe strain of virus X with virus A, in addition to mottling, puckering, and reduction in size of the leaflets, reduced the mean length of the leaf by from 2 to 3 inches, with corresponding reduction in yields of 46, 29, and 55 per cent., respectively, in Arran Banner, Majestic, and President.

Explanation of Plates.

All reduced to half size.

Plate 10.

Fig. 1. General appearance of healthy leaf of President.
Fig. 2. Leaf of President with Crinkle virus (X*c* + A).

Plate 11.

Fig. 3. Leaf of President with Crinkle virus (X*d* + A).
Fig. 4. Leaf of Arran Banner with Crinkle virus (X*d* + A).
Fig. 5. Leaf of Majestic with Crinkle virus (X*d* + A).

References.

1. Clinch, Phyllis E. M. and J. B. Loughnane.—Sci. Proc. R. Dub. Soc. **20** (N.S.), 567–596, 1933.

2. Clinch, Phyllis E. M. and Robert McKay.—Sci. Proc. R. Dub. Soc. **24** (N.S.), 189–198, 1947.

3. Clinch, Phyllis E. M. and Robert McKay.—Sci. Proc. R. Dub. Soc. **25** (N.S.), 93–99, 1949.

4. Loughnane, J. B. and P. A. Murphy.—Nature, **141**, p. 120, 15 January, 1938.

5. Murphy, P. A. and R. McKay.—Rapport 2 *me* Congrès Internat. de Pathologie Comparee, pp. 448–450, Paris, 1931.

6. Murphy, P. A. and R. McKay.—Sci. Proc. R. Dub. Soc. **20**, 227–247, 1932.

7. Smith, K. M. and Markham, R.—Nature, **155**, p. 38, 13 January, 1945.

Fig. 1

Fig. 2

Fig. 3

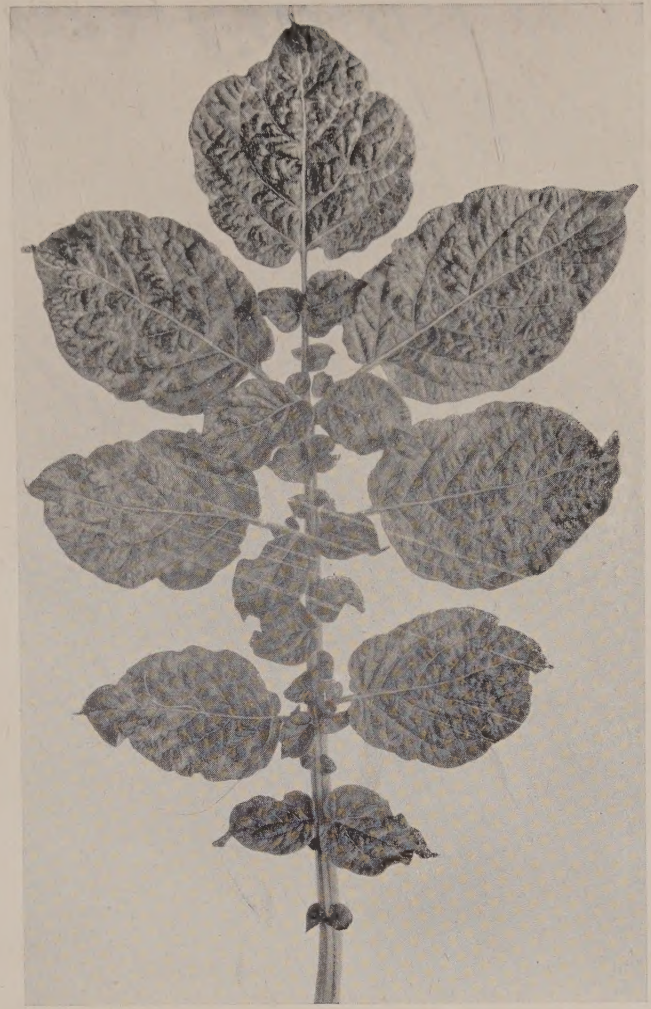

Fig. 4

Fig. 5